

Comparative physiology of salt and water stress

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ABSTRACT

Plant responses to salt and water stress have much in common. Salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate, along with a suite of metabolic changes identical to those caused by water stress. The initial reduction in shoot growth is probably due to hormonal signals generated by the roots. There may be salt-specific effects that later have an impact on growth; if excessive amounts of salt enter the plant, salt will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence, and reduce the photosynthetic leaf area of the plant to a level that cannot sustain growth. These effects take time to develop. Salt-tolerant plants differ from salt-sensitive ones in having a low rate of Na⁺ and Cl⁻ transport to leaves, and the ability to compartmentalize these ions in vacuoles to prevent their build-up in cytoplasm or cell walls and thus avoid salt toxicity. In order to understand the processes that give rise to tolerance of salt, as distinct from tolerance of osmotic stress, and to identify genes that control the transport of salt across membranes, it is important to avoid treatments that induce cell plasmolysis, and to design experiments that distinguish between tolerance of salt and tolerance of water stress.

Key-words: Plasmolysis; salinity; salt stress; water stress.

INTRODUCTION

Salinity affects 7% of the world's land area, which amounts to 930 million ha (Szabolcs 1994; based on FAO 1989 data). The area is increasing; a global study of land use over 45 years found that 6% had become saline (Ghassemi *et al.* 1995). This amounts to 77 million ha. In Australia alone, 2 million ha have become saline since clearing began a century ago, and another 15 million ha are at risk of becoming saline in the next 50 years (National Land and Water Resources Audit; <http://audit.ea.gov.au>). This represents a third of Australia's agricultural area. Irrigation systems are particularly prone to salinization; about half the existing irrigation systems of the world are under the influence of salinization, alkalization or waterlogging (Szabolcs 1994). Irrigation schemes cover only 15% of the cultivated land of the world (227 million ha in 1987), but as irrigated land has at least twice the productivity of rain-fed land, it may pro-

duce one-third of the world's food. Reducing the spread of salinization, and increasing the salt tolerance of high-yielding crops, are important global issues.

Salinization can be managed by changed farm management practices. In irrigated agriculture, better irrigation practices, such as drip irrigation, to optimize use of water can be employed. In rain-fed agriculture, practices such as rotation of annual crops with deep-rooted perennial species may restore the balance between rainfall and water use, thus preventing rising water tables bringing salts to the surface. All such practices will rely on a high degree of salt tolerance, not only of the perennial species used to lower a saline water table, but also of the crops to follow, as some salt will remain in the soil.

Salt tolerance is usually assessed as the percent biomass production in saline versus control conditions over a prolonged period of time. Dramatic differences are found between plant species. For example, after some time in 200 mm NaCl a salt-tolerant species such as sugarbeet might have a reduction of only 20% in dry weight, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might be dead (Greenway & Munns 1980). A halophyte such as *Suaeda maritima* might be growing at its optimum rate (Flowers *et al.* 1977, 1986). Salt tolerance can also be assessed in terms of survival, which is quite appropriate for perennial species, but for annual species, particularly for broadacre or horticultural crops, the rate of biomass production is more useful, as this usually correlates with yield.

It is surprisingly difficult to quantify differences in salt tolerance between closely related species, as the growth reduction depends on the period of time over which the plants have grown in saline conditions. During a short time in salinity, there will be a significant decrease in growth rate, but the decrease may be the same for species that have quite different reputations for salt tolerance. For example, durum wheat has the reputation of being more salt-sensitive than bread wheat, and its yield is more affected (Francois *et al.* 1986). Yet, over short periods of time in salinity, we have found no differences between durum and bread wheat cultivars, nor between barley and triticale cultivars (Munns *et al.* 1995). There were no significant differences between the leaf elongation rate in the first 10 d of salinization of any cultivar (Munns *et al.* 1995), including one that had proven to be the most sensitive (a durum wheat) and one (a barley) found to be the most tolerant (Rawson *et al.* 1988b). This led to consideration of time scale and the different mechanisms that may be important in controlling growth at different periods of time for plants exposed to salinity.

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IMPORTANCE OF TIME: WHEN DO SALT-SPECIFIC EFFECTS OCCUR?

When plants are exposed to salinity in laboratory experiments, there is a rapid and temporary drop in growth rate followed by a gradual recovery to a new reduced rate of growth. The temporary effects are clearly due to rapid and often transient changes in plant water relations. The subsequent changes in growth rate, and the underpinning molecular or metabolic events, are not so easily ascribed to water stress or to salt-specific effects. The next section considers these events over time, starting with a young plant growing in sand or solution culture that is exposed to salt.

Minutes to hours

In leaves there are rapid, essentially instantaneous, changes in growth rates with a sudden change in salinity (Fig. 1a). Rapid and transient reductions in leaf expansion rates after a sudden increase in salinity have been recorded in maize (Cramer & Bowman 1991; Neumann 1993), rice (Yeo *et al.* 1991) and wheat and barley (Passioura & Munns 2000). The same changes occur when KCl, mannitol or polyethylene glycol (PEG) are applied (Yeo *et al.* 1991; Chazen *et al.* 1995), showing that the responses are not salt-specific. The initial decrease in growth occurs so quickly and so transiently, and recovery is so rapid, that it must be solely due to changes in cell water relations (Fig. 1a; Yeo *et al.* 1991). This was confirmed by Passioura & Munns (2000) in experiments using a pressurization technique in which plants were maintained at maximum water status while the soil was salinized; the transient growth reduction when the salt was applied was prevented, and so was the transient surge when the salt was removed (Fig. 1b).

Several minutes after the initial decline of leaf growth, a gradual recovery is observed, which may continue for 30 min or more before reaching a new steady rate, as shown in Fig. 1(a). The time taken to recover, and the new steady rate, depend on the concentration of the salt solution (Cramer & Bowman 1991). Other osmotica such as KCl or mannitol, at the same osmotic pressures, have the same effect as NaCl (Yeo *et al.* 1991), indicating that the new reduced rate of growth is due to changed water relations and not the presence of Na^+ or Cl^- . Independent evidence was provided by the results of experiments using the pressurization technique mentioned above (Passioura & Munns 2000). Pressurization prevented not only the transient reduction in growth rate following the addition of NaCl, but there was no reduction in the steady growth rate (Fig. 1b). This also held for experiments run over many hours (Munns *et al.* 2000).

In roots, also, there are rapid and transient reductions in growth rates after sudden increases in NaCl (Rodríguez *et al.* 1997), and similar changes occur with KCl and mannitol (Frensch & Hsiao 1994, 1995), indicating that in roots as well as leaves these effects are entirely due to sudden changes in cell water relations. Responses very similar to those shown for leaves in Fig. 1(a) are presented by Frensch

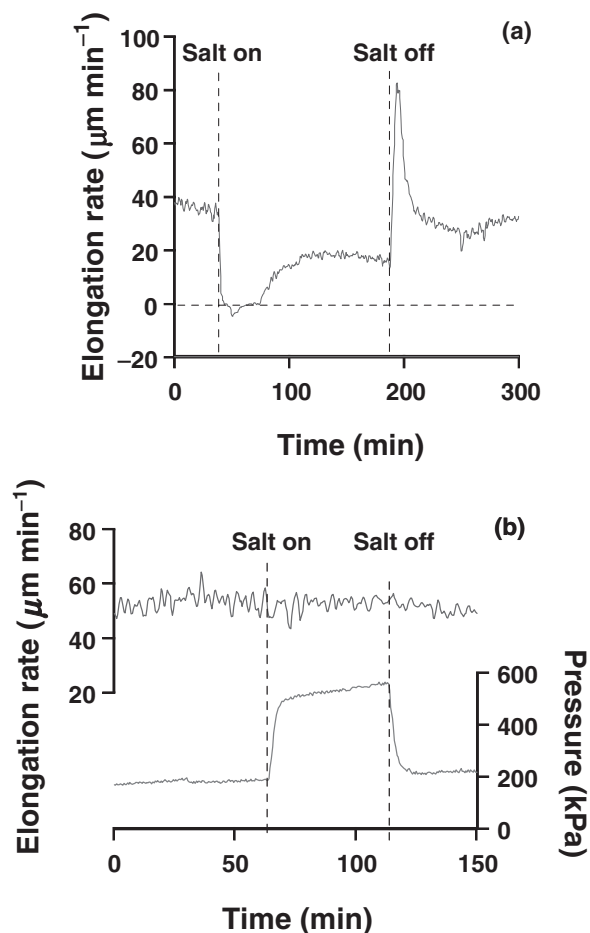


Figure 1. The effect of changes in salinity of soil solution on elongation rate of a barley leaf (a) with no control of leaf water status, and (b) with the plant maintained at balancing pressure, i.e. at constant leaf water status, throughout the changes. The vertical broken lines mark the times at which the light was turned on or off, and the broken horizontal line in (a) marks zero elongation rate. At the changes, full strength nutrient solution was exchanged with the same solution containing 75 mM NaCl or vice versa. Reproduced from Passioura & Munns (2000), with permission of the publisher.

(1997) for roots of maize plants using 0.3 MPa of mannitol as an osmoticum.

Root growth, in contrast to leaf growth, recovers remarkably well from the addition of salt or other osmotica (Hsiao & Xu 2000). With moderate levels of osmotic stress (0.1–0.4 MPa of mannitol or KCl), the recovery of growth by maize roots was essentially complete within 1 h (Frensch & Hsiao 1994, 1995). With a larger osmotic shock (0.6 MPa of mannitol or KCl) there was little or no recovery in elongation rate within 1 h (Frensch & Hsiao 1995), but it is possible that recovery could have occurred later. Rodríguez *et al.* (1997) found that growth of maize roots treated with NaCl as high as 150 mM (0.7 MPa) recovered fully after 24 h. The time taken for the roots to recover may depend on whether or not plasmolysis has occurred. This issue is discussed in more detail later.

A salt-specific effect on root growth can occur: a salt-induced Ca^{2+} deficiency that is more pronounced for genotypes with low uptake rates for Ca^+ . Salts in the nutrient solution lower the activity of Ca^{2+} (Cramer & Läuchli 1986), and root growth can be severely and quickly affected. For example, root elongation of maize was not affected by the addition of 80 mM NaCl, provided that supplemental Ca^{2+} (10 mM) was given. When it was not, there was a reduction in root growth that was not readily reversed by subsequent supply of Ca^{2+} (Cramer *et al.* 1988). Even at 150 mM NaCl, elongation of sorghum roots was reduced by only 20% if supplemental Ca^{2+} was present, but by 80% if it was not (Colmer *et al.* 1996).

Days

By this time, leaf and root growth have settled down to a reduced steady rate. Leaf growth is often more reduced than root growth by salinity, a phenomenon in common with dry soil (Hsiao & Xu 2000; Munns & Sharp 1993), the commonality indicating this is probably due to factors associated with water stress rather than a salt-specific effect. This is supported by the evidence that Na^+ and Cl^- are always below toxic concentrations in the growing cells themselves. For example, Hu & Schmidhalter (1998) showed that in wheat growing in 120 mM NaCl, with a 25% reduction in growth rate, Na^+ in the growing cells of leaves was only 20 mM at maximum, and Cl^- only 60 mM (Fig. 2). K^+ was maintained at high levels (Fig. 2). In roots, also, there is evidence that Na^+ concentrations in dividing or rapidly elongating cells are low and well below toxic levels (Jeschke 1984; Jeschke *et al.* 1986). For example, in root tips of saltbush (*Atriplex amnicola*), Na^+ was only 40 mM at external NaCl concentrations of 400 mM (Jeschke *et al.* 1986). The rapid expansion of the growing cells would help to stop the salt building up to high concentrations.

Whether water status, hormonal regulation or supply of photosynthate exerts the dominant control over growth of plants in dry or saline soil is an issue that has been debated for the last two decades, and not yet resolved. Over the timescale of days, there is much evidence to suggest that hormonal signals rather than water relations are controlling growth in saline soils. The evidence for this is that leaf expansion over 24 h of plants in saline soil does not respond to an increase in leaf water status. These experiments were done by growing plants in sand in pots that could be placed in pressure chambers, watering with saline solution, and then pressurizing the chambers with a pressure equal to the osmotic pressure of the solution, which was usually 100 mM NaCl. No lasting effect of pressurization on growth was found over periods up to 8 d, with species as diverse as barley and wheat (Termaat *et al.* 1985), white lupin and Egyptian clover (Munns & Termaat 1986), as well as a halophyte, the saltbush *Atriplex spongosa* (Munns 1993). More recently, pressurization was done at balancing pressures, with barley and maize, so that the leaf water potential was maintained close to its maximum both day or night, while the soil was watered with 100 mM NaCl. This treatment,

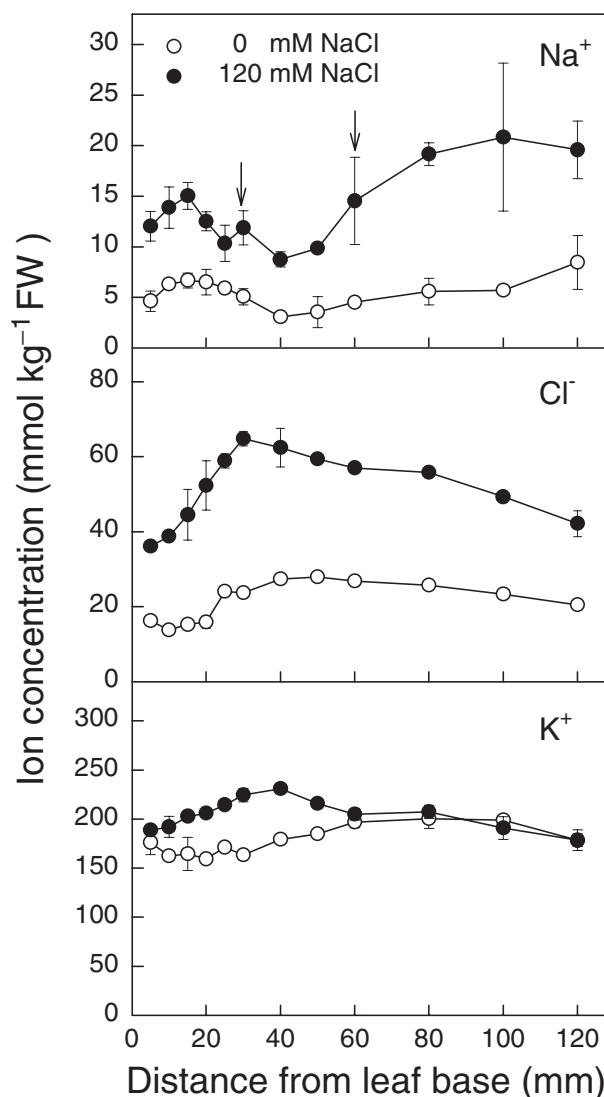


Figure 2. Spatial distributions of Na^+ , Cl^- and K^+ concentrations in the growing leaf 4 on the mainstem of wheat grown in soil with 0 and 120 mM NaCl. Plants were harvested at 3 h into a 16 h photoperiod. Arrows indicate the end of the growth zone and leaf sheath. Error bars ($n = 2$) present standard errors and fit within the plot symbol if not otherwise shown. Adapted from Hu & Schmidhalter (1998).

also, failed to make plants grow faster over the time scale of 24 h (Munns *et al.* 2000), indicating that hormonal signals, and not leaf water deficit or ion toxicity, were controlling growth. The results were reminiscent in every way of experiments done with plants in dry soil; these had showed no response of leaf growth to an increase in shoot water status brought about by pressurization at balancing pressures (Passioura 1988). Furthermore, 'split-root' experiments with plants whose root systems were divided between wet and dry soils showed that leaf expansion decreased while leaf water status was unaffected (Saab & Sharp 1989; Gowing *et al.* 1990).

In very salt-sensitive species, salt-specific effects can

become visible after several days at high salinities. If the salinity is high, and if the plant has a poor ability to exclude NaCl, marked injury in older leaves might occur within days, as found for white lupin once the salinity increased above 100 mM NaCl (Munns 1988). Salt injury is due to Na⁺ or Cl⁻ (or both) accumulating in transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize these ions in the vacuole. Ions then build up rapidly in the cytoplasm and inhibit enzyme activity, or they build up in the cell walls and dehydrate the cell (Flowers & Yeo 1986; Munns & Passioura 1984).

The consequences of Na⁺ or Cl⁻ build-up in the cell walls are catastrophic. As the concentration in the wall starts to rise, the cell will shrink, the concentration of ions inside will rise, and some will efflux, adding to those still arriving in the transpiration stream from the roots. The ion concentration in the wall will rapidly escalate, and the cell will rapidly dehydrate (Munns & Passioura 1984; their Fig. 1). If Na⁺ or Cl⁻ do not increase in cell walls when the vacuole becomes 'full', they must build up in the cytoplasm. This would be equally catastrophic (Munns 1993; their Fig. 4). The rate at which the concentration increases in the cytoplasm would be much greater than the rate it increased in the vacuole, because the volume of the cytoplasm is so much smaller than that of the vacuole. For example, if Na⁺ is accumulating at the rate of 5 mM (leaf water basis) per day, a rate that is typical for a reasonably salt-tolerant temperate species (e.g. barley; Rawson *et al.* 1988a), and the cytoplasmic volume is a tenth of the vacuolar volume, then Na⁺ would increase in the cytoplasm at 50 mM (cytoplasmic water basis) per day. In 2 d the salt concentration in the cytoplasm would exceed 100 mM, and so be potentially toxic to many enzymes (Munns *et al.* 1983). The cell thus dies of salt poisoning or dehydration, depending on whether the ions build up in the cytoplasm or cell wall. In either case, the cell would die within a few days of the vacuole ceasing to take up incoming salt.

Salt toxicity is seen in older rather than younger leaves, i.e. the leaves that have been transpiring the longest. Na⁺ and Cl⁻ increase with time in any given leaf (e.g. Jeschke & Wolf 1985), and are higher in older than younger leaves at any given time (e.g. Colmer *et al.* 1995). Salts will eventually build up to excessive concentrations in the transpiring leaves, but whether this occurs in days or weeks depends on the salinity level and other environmental conditions such as temperature, relative humidity, composition of the nutrient solutions, and genetic differences in the ability of roots to keep salt out of the transpiration stream arriving in the leaves.

Weeks

The effects of salinity may become obvious over weeks, especially in the more sensitive species, that is, those with high rates of salt uptake or inability to compartmentalize salt once it builds up in leaves. The injury may be visible as yellowing or death of older leaves. In the more salt-tolerant species, in which the rate of salt uptake is low, or which have

the ability to compartmentalize the salt efficiently in cell vacuoles and so prevent it building up in the cytoplasm or cell wall, most developmental events and metabolic activities are common to plants under water stress.

In the salt-sensitive species, in which salt is not effectively excluded from the transpiration stream, salt will have built up to toxic levels in the leaves that have been transpiring the longest. This results in a progressive loss of the older leaves with time. The rate at which they die becomes the crucial issue determining the survival of the plant. If new leaves are continually produced at a rate greater than that at which old leaves die, then there is enough photosynthetic surface for the plant to enter into the reproductive phase. However, if old leaves die faster than new leaves are produced, then the proportion of leaves that are injured starts to increase, and the number of green and healthy leaves will ultimately decline. There is then a race against time to initiate flowers and produce seeds while there is still an adequate number of green leaves left to supply the necessary photosynthate.

Months (and reproductive development)

In perennials, progressive leaf death will continue. Whether the plant lives or dies depends on its ability to prevent salt from reaching toxic levels in the older leaves (determined by the degree of exclusion from the transpiration, and the ability to compartmentalize the salt in vacuoles) and the rate of new leaf growth (which is determined by the soil water potential).

In annuals, salinity affects the formation and viability of reproductive organs. In cereals, it reduces the number of florets per ear, and alters the time of flowering and hence maturity (e.g. Munns & Rawson 1999). Similar phenomena occur under drought. A salt-specific effect is not a likely cause of the altered reproductive development; the levels of Na⁺ and Cl⁻ present in the reproductive primordia are too low to affect metabolism. For example, in the reproductive apex of a salt-sensitive cultivar of barley during ear development, Na⁺ increased to a maximum of 50 mM at the time of final spikelet initiation, whereas Cl⁻ remained low at 10–15 mM, and similar results were found with four other barley and wheat cultivars with known differences in salt tolerance (Munns & Rawson 1999). Most cells in the apex are essentially unvacuolated, so any salt arriving there would accumulate in the cytoplasm. It appears that transport of Na⁺ and Cl⁻ in the phloem to the apex is sufficiently well controlled to prevent concentrations of these ions reaching toxic levels in these cells.

Summary of time-dependent changes

Table 1 summarizes the sequence of events in a plant when exposed to salinity. In the first few seconds or minutes, cells lose water and shrink. Over hours, cells regain their original volume but cell elongation rates are reduced, leading to lower rates of leaf and root growth. Over days, changes in cell elongation and cell division lead to slower leaf appear-

Table 1. Plant response to salinity at different time scales. The effects on a salt-tolerant plant are basically identical to those due to soil water deficit

Time	Water stress effects	Salt-specific effects
	(Observed effect on growth of a salt-tolerant plant)	(Additional effects on growth of a salt-sensitive plant)
Minutes	Instant reduction in leaf and root elongation rate then rapid partial recovery	
Hours	Steady but reduced rate of leaf and root elongation	
Days	Leaf growth more affected than root growth; Reduced rate of leaf emergence	Injury visible in oldest leaf
Weeks	Reduced final leaf size and/or number of lateral shoots	Death of older leaves
Months	Altered flowering time, reduced seed production	Younger leaves dead, plant may die before seed matures

ance and smaller final size, and leaf growth is usually more affected than root growth. In plants with high salt uptake rates, the oldest leaf may start to show symptoms of injury. After weeks it is clear that lateral shoots have been inhibited, and in plants with high salt uptake rates a number of leaves may be dead. However, the rate of production of younger leaves may not yet differ between genotype. After months, differences between plants with high and low salt uptake rates become very apparent, with a large amount of leaf injury and complete death in some cases if the salinity level is high enough.

TWO-PHASE GROWTH RESPONSE

Recognition of the importance of time frame led to the concept of a two-phase growth response to salinity (Munns 1993). This is very important when screening plants for salt tolerance. The first phase of growth reduction is quickly apparent, and is due to the salt outside the roots. It is essentially a water stress or osmotic phase, for which there is surprisingly little genotypic variation. The growth reduction is presumably regulated by hormonal signals coming from the roots. Then there is a second phase of growth reduction, which takes time to develop, and results from internal injury. It is due to salts accumulating in transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize salts in the vacuole. This will inhibit growth of the younger leaves by reducing the supply of carbohydrates to the growing cells.

The two-phase growth response has been shown clearly for maize and wheat cultivars. Two maize cultivars with two-fold differences in rates of Na^+ accumulation in leaves had the same growth reduction for 15 d in 80 mM NaCl (Cramer *et al.* 1994). Furthermore, another two maize cultivars, again with two-fold differences in Na^+ accumulation, had the same growth reduction for 4 weeks in 100 mM NaCl, and it was not until 8 weeks that a growth difference was clearly seen (Fortmeier & Schubert 1995). Similar results were found in wheat (Munns *et al.* 1995). Two closely related wheat genotypes that differed in rates of Na^+ accumulation had the same growth reduction for the first 4 weeks in 150 mM NaCl, and only in the fifth and sixth week

were there differences in dry weight production between the genotypes (Fig. 3). However, visual differences appeared long before that; the genotype with the higher rate of Na^+ uptake showed faster leaf senescence, with injury appearing in the oldest leaf after 2 weeks (Fig. 3). Later, when the proportion of dead leaves increased above about 20% of the total, the rate of new leaf production slowed down dramatically, and some individuals died. These data illustrate the principle that the initial growth reduction is due to the osmotic effect of the salt outside the roots, and that what distinguishes a salt-sensitive plant from a more tolerant one is the inability to prevent salt from reaching toxic levels in the transpiring leaves.

With rice, also, a clear distinction has been made between the initial effects of salinity, which are recoverable,

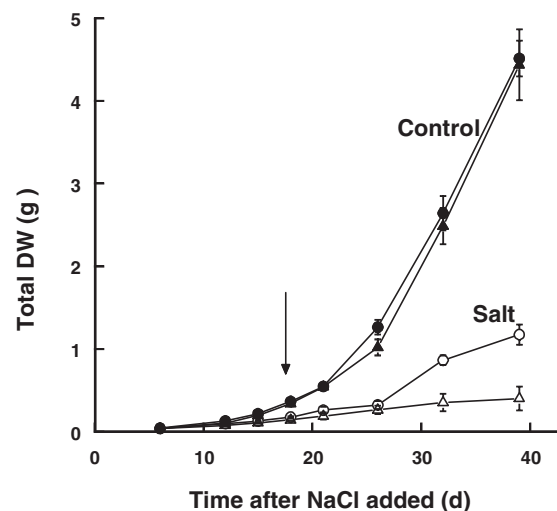


Figure 3. Two accessions of the diploid wheat progenitor *Triticum tauschii* in control solution (closed symbols) and in 150 mM NaCl with supplemental Ca^{2+} (open symbols). Circles denote the tolerant accession, triangles the sensitive one. The arrow marks the time at which symptoms of salt injury could be seen on the sensitive accession; at that time the proportion of dead leaves was 10% for the sensitive and 1% for the tolerant accession (Munns *et al.* 1995). A similar result is given in Fortmeier & Schubert (1995).

and the long-term effects that result from the accumulation of salt within expanded leaves (Yeo *et al.* 1991).

MECHANISMS OF SALT TOLERANCE

Underlying all mechanisms is the early discovery by biochemists that enzymes of halophytes (plants adapted to saline habitats) are no more tolerant of high concentrations of NaCl than are those of non-halophytes (also called glycophytes, or plants adapted to sweet water). For example, *in vitro* activities of enzymes extracted from the halophytes *Atriplex spongiosa* or *Suaeda maritima* were just as sensitive to NaCl as were those extracted from beans or peas (Greenway & Osmond 1972; Flowers *et al.* 1977). Even enzymes from the pink salt-lake alga *Dunaliella parva*, which can grow at salinities 10-fold higher than those of seawater, are as sensitive to NaCl as those of the most sensitive glycophytes (reviewed by Munns *et al.* 1983). Generally, Na⁺ starts to inhibit most enzymes at a concentration above 100 mM. The concentration at which Cl⁻ becomes toxic is even less well defined, but is probably in the same range as that for Na⁺. Even K⁺ may inhibit enzymes at concentrations of 100–200 mM (Greenway & Osmond 1972).

Mechanisms for salt tolerance are therefore of two main types: those minimizing the entry of salt into the plant, and those minimizing the concentration of salt in the cytoplasm. Halophytes have both types of mechanisms; they 'exclude' salt well, but effectively compartmentalize in vacuoles the salt that inevitably gets in. This allows them to grow for long periods of time in saline soil. Some glycophytes also exclude the salt well, but are unable to compartmentalize the residual salt taken up as effectively as do halophytes. Most glycophytes have a poor ability to exclude salt, and it concentrates to toxic levels in the transpiring leaves

Low salt transport to leaves – the mechanism known as 'salt exclusion'

'Salt exclusion' functions to reduce the rate at which salt accumulates in transpiring organs. Plants transpire 30–70 times more water than they use for cell expansion, the value depending largely on the prevailing weather. This means that solutes in the soil that are not excluded by roots will be 30–70 times more concentrated than in the soil solution. This concentration can be avoided by filtering out most of the salt. For example, if a plant is transpiring 50 times more water than it retains, and lets in only 2% of the salt in the soil solution (i.e. excludes 98%), the concentration of salt in the shoot as a whole would never increase over that in the soil and the plant could grow indefinitely in saline soil.

Individual leaves, however, cannot be protected indefinitely. Salts carried in the transpiration stream are deposited in leaves as the water evaporates, and salt gradually builds up with time. The salt concentrations in older leaves are therefore much higher than in younger leaves, at any one point in time. In the older leaves, the salt concentration eventually becomes high enough to kill the cells.

The mechanisms by which salt is excluded from leaves are:

- 1 Selectivity of uptake by root cells. It is still unclear which cell types control the selectivity of ions from the soil solution. The initial uptake of Na⁺ and Cl⁻ could occur at the epidermis, at the exodermis, or if soil solution flows apoplastically across the root cortex, it would occur at the endodermis.
- 2 Loading of the xylem. There is evidence for a preferential loading of K⁺ rather than Na⁺ by the stellar cells that is under genetic control (Gorham *et al.*, 1990).
- 3 Removal of salt from the xylem in the upper part of the roots, the stem, petiole or leaf sheaths. In many species, Na⁺ is retained in the upper part of the root system and in the lower part of the shoot, indicating an exchange of K⁺ for Na⁺ by the cells lining the transpiration stream.

Mechanisms conferring exclusion that operate at the cellular and whole plant level have been reviewed by Greenway & Munns (1980), Läuchli 1984), Storey & Walker (1999), and with particular reference to selectivity for K⁺ over Na⁺, by Jeschke (1984). Some glycophytes control the transport of salt to the leaves reasonably well, at least at low to moderate salinities. For example, the Na⁺ and Cl⁻ in the xylem in barley at 100 mM was only about 5 mM, i.e. 95% was excluded (Munns 1985). Salt exclusion is also the most important adaptive feature regulating the internal salt load of halophytes, even in species that have salt glands or bladders. As an example, the salt concentration in the xylem sap of the mangrove *Avicennia marina* was calculated as only 9 mM for plants growing in 500 mM NaCl, a degree of salt exclusion of 98% (Ball 1988). Exclusion is particularly important for perennials, the leaves of which may live for a year or more; there is greater need to regulate the incoming salt load over a much longer period of time than for annual species, whose leaves may live for only one month.

Evidence for the importance of these processes in the control of salt accumulation in leaves, and in determining the salt tolerance of a number of species, can be gained from the publications cited above. The electrophysiology of the ion channels and transporters that work together to regulate the net movement of salt across cell membranes is well understood, and genes for these channels and transporters are being identified. The status of this research at the electrophysiological and molecular level has been described comprehensively in several recent reviews (Amtmann & Sanders 1999; Tyerman & Skerrett 1999, Schachtman & Liu 1999; Hasegawa *et al.* 2000).

There are contributory features that function to maintain low rates of salt accumulation in leaves. High shoot : root ratios and high intrinsic growth rates will reduce the rate at which salt enters the transpiration stream and accumulates in the shoot (Pitman 1984), and the extent of an apoplastic pathway in roots will also influence the movement of salts across the root and to the xylem (Garcia *et al.* 1997).

Export from leaves in the phloem is another contribu-

tory feature that can help to maintain low salt concentrations. However, there appears to be relatively little retranslocation of salt from leaves, in relation to the import in the transpiration stream. This can be seen in the continued presence of salt in leaves long after the salt around the roots is removed. Measurements of ions in phloem sap have indicated that the more salt-tolerant species exclude Na^+ and Cl^- from the phloem to a large extent, whereas less tolerant ones do not have the same restriction (Munns *et al.* 1988 and references therein). Exclusion of salt from the phloem ensures that salt is not redirected to growing tissues of the shoot. Another mechanism of export from leaves is excretion through salt glands or bladders. These can help to maintain a steady salt balance in leaves over long periods of time (Ball 1988). These specialized cell types are confined to halophytes (Flowers *et al.* 1977, 1986)

Intracellular ion compartmentation

Ideally, Na^+ and Cl^- should be sequestered in the vacuole of the cell. That this occurs in most species is indicated by the high concentrations found in leaves that are still functioning normally. Concentrations well over 200 mM are common, yet these same concentrations will severely inhibit enzyme activity *in vitro*, as mentioned above. Obtaining direct experimental evidence for compartmentation is technically difficult, but X-ray microanalysis of cultured tobacco cells growing in 430 mM NaCl indicated that Na^+ and Cl^- concentrations in the vacuole were about 780 and 625 mM, respectively, whereas cytoplasmic concentrations of both ions were below 100 mM (Binzel *et al.* 1988).

If Na^+ and Cl^- are sequestered in the vacuole of a cell, K^+ and organic solutes should accumulate in the cytoplasm and organelles to balance the osmotic pressure of the ions in the vacuole. The compounds that accumulate most commonly are proline and glycine betaine, although other molecules can accumulate to high concentrations in certain species (Hasegawa *et al.* 2000). All these compounds accumulate under water stress as well as salt stress, and are found at high concentrations in plants adapted to dry or saline soils. Their role in balancing intracellular ion compartmentation is not clear. Their accumulation in the leaf can be so high that it clearly is not confined to the cytoplasmic compartments. Furthermore, their level of accumulation relates more to the osmotic stress than any specific salt effect. For instance, the accumulation of proline and glycine betaine was similar in barley treated with PEG and NaCl at the same osmotic pressures (Wyn Jones & Storey 1978a).

OSMOTIC ADJUSTMENT, ENERGY COSTS AND GROWTH

In relation to the comparative physiology of osmotic adjustment in saline versus dry soil, there is the issue of metabolic costs for osmotic adjustment. Growing or surviving in a saline soil imposes some costs; the cost of excluding salt, of intracellular compartmentation, and of excreting it through

salt glands. This cost, however, is relatively small in relation to that needed to synthesize organic solutes for osmotic adjustment (Yeo 1983; Raven 1985). The number of moles of ATP needed to use one mole of NaCl as an osmoticum is approximately 4 in root cells, and 7 in leaf cells, whereas the number required to synthesize an organic compound is an order of magnitude higher (Raven 1985). The ATP requirement for the synthesis or accumulation of solutes in leaves was assessed by Raven (1985) as 3.5 for Na^+ , 34 for mannitol, 41 for proline, 50 for glycine betaine and about 52 for sucrose. (These values assume a production of 0.5 mole of ATP per photon, and nitrate as the source of N).

However, it is not clear that plants produce fewer organic solutes when growing on saline than on non-saline soil of the same water potential. As mentioned above, the accumulation of proline and glycine betaine was similar in barley treated with PEG and NaCl at the same osmotic pressures (Wyn Jones & Storey 1978a). This might be restricted to nitrogen-containing or methylated compounds, as a comparison of four tomato genotypes showed much greater accumulation of soluble sugars in leaves of plants grown in PEG than in NaCl at the same osmotic pressure, but a similar accumulation of amino acids (Pérez-Alfocea *et al.* 1993).

If accumulating organic solutes demands more energy than accumulating ions, it might follow that plants grow faster in saline than dry soils of the same water potential. However, there is little evidence for this. Attempts to compare the growth of plants in NaCl with those in PEG or other osmotica have produced equivocal results. Comparisons between NaCl and PEG at the same osmotic pressure showed that barley grew better in PEG than NaCl (Storey & Wyn Jones 1978); however, various tomato genotypes grew the same or worse in PEG than NaCl (Pérez-Alfocea *et al.* 1993). Comparisons between NaCl and concentrated mixed salts (not containing NaCl) at the same osmotic pressure showed that barley grew better in the medium without NaCl (Termaat & Munns 1986), as did bean (Montero *et al.* 1998). However, all these comparisons are compromised by lack of supplemental Ca^{2+} , and the likelihood of a NaCl-induced Ca^{2+} deficiency. As explained earlier, the presence of salt will lower the activity of Ca^{2+} , PEG will not, and the mixed salts were in fact concentrated Hoagland's solution, and so included an increased concentration of Ca^{2+} .

THE ENIGMA OF ROOTS

Little work has been done on roots with regard to either salt or water stress. Roots might seem the part of the plant most vulnerable as they are directly exposed to salt or to drying soil, but they are surprisingly robust. As shown earlier, their growth rate is not as affected as that of shoots. Their ionic status is relatively good; their ion concentrations do not increase with time, as in leaves, and they often have a lower Na^+ and Cl^- concentration than the external solution, which rarely happens in leaves. For example, in wheat growing in 150 mM NaCl, Na^+ in the roots was only

20–40 mM, the variation being due to different genotypes (Gorham *et al.* 1990). We have found similarly low values for both Na^+ and Cl^- , with a range of wheat genotypes and salinities from 50 to 150 mM NaCl (S. Husain and R. Munns, unpublished results). K^+ did not make a large contribution. This raises the interesting question of organic solutes for osmotic adjustment, as K^+ is often lower in roots than shoots (e.g. Gorham *et al.* 1990). However, there is no evidence that organic solutes are more likely to accumulate in roots; on the contrary, they are often lower in roots than shoots. For example, proline and glycine betaine concentrations on a fresh weight basis were five times lower in roots than shoots of barley over 100–200 mM NaCl, even though the Na^+ and Cl^- concentrations were much lower in roots than shoots (Wyn Jones & Storey 1978b). The authors reported similar concentrations in response to PEG (Wyn Jones & Storey 1978a). As osmotic adjustment is presumably as important for roots as shoots, this suggests that unknown solutes are involved.

There is surprisingly little known about the metabolic regulation of osmotic pressure in relation to organic solute synthesis and compartmentation in response to salinity, either in shoots or roots.

OSMOTIC SHOCK AND PLASMOLYSIS – IMPLICATIONS FOR GENE DISCOVERY

In laboratory experiments involving salinity, it is difficult to avoid giving plants an osmotic shock of some degree; the osmotic pressure of the solution around the roots can be changed so quickly. It is easy to give a shock large enough to cause a complete cessation of growth, and a loss of water from all cells.

The turgor of young root cells is normally around

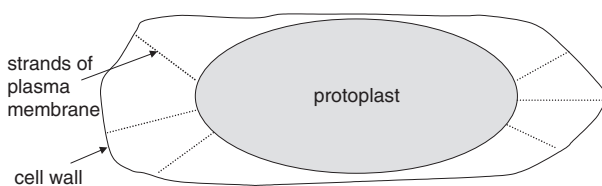


Figure 4. Plasmolysis in root cells. Diagram shows an epidermal cell of barley roots 10 min after transfer to 200 mM NaCl, showing the protoplast (grey) pulled away from the ends of the cell. The volume reduction of the protoplast is about 50%. The shrinkage can be estimated by assuming that the turgor of the root cells is about 0.5 MPa (e.g. Pritchard *et al.* 1991), that the cells are in pure water, and so the osmotic pressure of the cells is also 0.5 MPa. If 100 mM NaCl (about 0.5 MPa) were added in one step, the cell would lose water until the external osmotic pressure equalled the internal osmotic pressure, turgor would be close to zero, and plasmolysis imminent. With a higher concentration of NaCl, the loss of water would continue until the internal osmotic pressure again equalled the external, even though the plasma membrane would detach from large sections of the wall. With 200 mM NaCl, the protoplast would shrink to half its original volume to achieve equilibrium and solution would occupy the space between the plasma membrane and the cell wall.

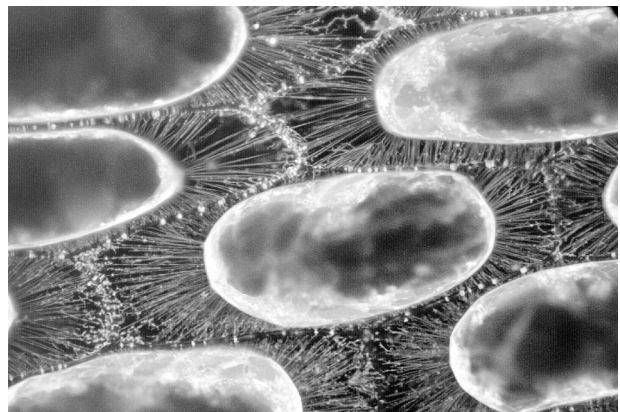


Figure 5. Onion scale leaf epidermal cells plasmolysed in 0.75 M mannitol. Stretched strands of plasma membrane connect the protoplast to the cell wall; small sections of the plasma membrane and peripheral cytoplasm remain tethered to the wall. Confocal projection, $\sim \times 750$. Photograph courtesy of Brian Gunning.

0.6 MPa. Turgor pressures in the range of 0.5–0.7 have been measured with a pressure probe in maize (Frensch & Hsiao 1994) and wheat (Pritchard *et al.* 1991), the variation possibly being associated with the developmental stage of the cells and the growing conditions of the plants. The osmotic pressure of a solution of 150 mM NaCl is about 0.7 MPa, so cells with a turgor less than this will plasmolyse if the plants are transferred in one step to 150 mM NaCl. If cell turgor is only 0.4 MPa, which is likely for older cells in roots (Pritchard *et al.* 1991) then a one-step transfer to a solution of 100 mM NaCl (0.5 MPa) will plasmolyse cells. (This assumes that the new solution also contains the same nutrient concentration as the growth solution.)

Plasmolysis occurs in cells that are in contact with solution, such as in epidermal strips, or in root cells of intact plants growing hydroponically. Plasmolysis starts when the osmotic pressure of the solution is increased above that of the cells, causing the protoplast to shrink, and the plasma membrane separates from the wall (Fig. 4). Large gaps created between the plasma membrane and the wall may fill with solution and allow an artefactual apoplastic pathway for salts to move radially across the root (Fig. 4&5). During this time, the plasma membrane is stretched into strands that remain tethered to the wall at particular sites (Fig. 5). Plasma membranes and plasmodesmata can be repaired, but in the meantime, an unregulated flux of solutes in or out of the protoplast occurs. It is likely that signal transduction pathways that arise from calcium or proton fluxes across the plasma membrane may not be functioning normally.

The degree of damage, and the time for recovery, depends on the degree of the initial change. This can be gauged from the effects on root growth, as mentioned earlier. With the addition of 0.1–0.4 MPa (mannitol or KCl), the recovery of growth by maize roots was essentially complete within 1 h, but with a larger osmotic shock (0.6 MPa) there was little or no recovery in elongation rate within 1 h (Frensch & Hsiao 1994, 1995). Root growth of wheat after

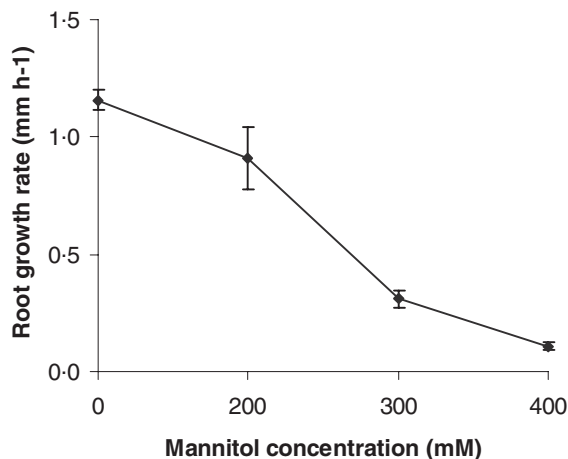


Figure 6. Growth of wheat roots at 24 h after sudden addition of mannitol (rate averaged over 18 and 30 h after transfer). The osmotic pressure of 200 mM mannitol is 0.5 MPa, so plasmolysis would have occurred at the higher concentrations (see legend of Figure 4). Data from Pritchard *et al.* (1991).

addition of 200 mM mannitol (similar in osmotic pressure to 100 mM NaCl) was still reduced a little after 24 h (Fig. 6), but not to the degree after treatment with 300 or 400 mM mannitol (Fig. 6), the latter presumably causing plasmolysis (Pritchard *et al.* 1991). There might have been recovery with longer periods of time: Rodríguez *et al.* (1997) found that growth of maize roots treated with 100 mM NaCl recovered fully after 24 h, and after 36 h with 150 mM NaCl. These authors also compared the effects of a salt shock versus a gradual increase, and found that salt had no effect on root growth at concentrations up to 100 mM NaCl as long as the concentration was increased gradually (Rodríguez *et al.* 1997). The deleterious effects of a salt shock are presumably associated with the plasmolysis of root cells.

Gene expression varies with the time after the salt shock is applied. After rice was suddenly exposed to 150 mM NaCl, the genes expressed in roots at 15 min were different from those expressed after 1 week (Kawasaki *et al.* 2001). It is likely that many genes induced soon after salt is applied are related to water stress rather than specifically to salt stress. In wheat roots, a gene expressed in the early phase of the stress response, 6 h after sudden exposure to 250 mM NaCl, was identified as a protein kinase, and induced by abscisic acid as well as by salinity (Shen *et al.* 2001). Whether these changes in gene expression relate to acclimation to long-term tolerance of salinity or of water stress remains to be investigated.

Leaf cells will not suffer the same trauma as roots after an osmotic shock. If the cells are not in contact with the plasmolysing solution, and still surrounded by air as normal, the wall will collapse around the shrinking protoplast in the phenomenon known as 'cytorrhesis' (Carpita *et al.* 1979). The integrity of the cell wall is affected, but the plasma membrane remains attached, and so there is presumably less trauma to the plasma membrane than with plasmolysis. This also applies to root cells in drying soil, or

when dehydrating on a laboratory bench. The effect of plasmolysis versus cytorrhesis on membrane transport processes and their recovery was investigated in *Chara corallina* for HCO_3^- and OH^- transport (Lucas & Alexander 1981). When cells were plasmolysed with sorbitol, mannitol or sucrose, all transport activity was lost and did not recover when cells were transferred back to control solution. In contrast, when cells were air-dried to zero turgor and re-wetted, transport activity fully recovered.

Studies on signal transduction pathways that are initiated by salt or water stress could exploit the phenomena of plasmolysis and cytorrhesis to distinguish between processes induced by membrane damage versus those induced by the stress itself.

The damaging effects are not confined to the first few hours or days after salt is imposed. Effects on salt accumulation and growth can be seen weeks afterwards. In a comparison of wheat plants undergoing gradual versus sudden applications of NaCl, higher shoot Na^+ concentrations and greater reductions in growth rates occurred when the application of NaCl was sudden rather than gradual (Almansouri *et al.* 1999). It is possible that the abnormal amounts of salt reached the shoots via apoplastic pathways opened by plasmolysis. This was indicated by a study of Storey & Wyn Jones (1978) on growth and ion relations of barley in 250 mM NaCl when the application was sudden rather than gradual. Within 2 d of the sudden shock, shoots contained Na^+ and Cl^- concentrations of $3.5 \text{ mmol g}^{-1} \text{ DW}$, compared to only $1.4 \text{ mmol g}^{-1} \text{ DW}$ when the salt was applied gradually. There was a very large efflux of K^+ from roots, the root concentration dropping four-fold. The extra salt transported to the shoot may exert a toxic effect for some time – once the salt has reached the shoot, there may be little opportunity for it to be removed.

A sudden shock with a non-ionic osmoticum can be just as deleterious as a salt shock. Barley plants exposed to PEG of equivalent osmotic pressure as 250 mM NaCl rapidly wilted and did not survive 4 d, whereas those exposed to 250 mM NaCl recovered their turgidity after 2 d and did survive (Storey & Wyn Jones 1978).

In summary, plasmolytic trauma combines a sudden removal of water with a leaky membrane letting in abnormal amounts of salt into the root cell, or opening an apoplastic pathway for abnormal amounts of salt to move across the shoot. Treatments that cause it should be avoided where possible.

CONCLUDING REMARKS

This paper has concentrated on cell water relations and growth, and shows that the early responses to water and salt stress are essentially identical. Salt-specific effects occur mainly in old leaves where salt brought in with the transpiration stream accumulates to high levels over time.

The similarity between water and salt stress also apply to most metabolic processes: all processes apart from those relating to ion transport. Hormonal responses are similar;

for instance, abscisic acid levels rise within 1 h of a imposition of water stress (Bensen *et al.* 1988) and salt stress (He & Cramer 1996). Photosynthesis also decreases in both water and salt stress, the responses being over the time scale of days rather than minutes. Although there can be strong correlations between increases in leaf ion concentrations and reductions in photosynthesis or stomatal conductance, there is as yet no unequivocal evidence for causal relationships. Correlations can disappear when considering different leaves, or different salinities (Rawson *et al.* 1988a). Experiments using different genotypes differing in rates of Na⁺ or Cl⁻ accumulation may be able to distinguish between the effects of salt in the leaf, and salt in the soil. Alternatively, stress-relief experiments can show whether the salt in the leaf is causing the decrease in stomatal conductance or photosynthesis, as leaching the soil will quickly restore the water relations of the plant but not affect salt levels in leaves. Such experiments with olive trees have indicated that the decreases in photosynthesis were due to the osmotic effect of the salt outside the roots, not a specific effect of the salt in the leaves (Tattini *et al.* 1995).

Understanding the mechanisms operating at the whole plant level has implications for screening techniques to distinguish plants that are tolerant of salinity as distinct from soil drying. Salinity can affect growth in a number of ways. The first phase of the growth response is due to the osmotic effect of the salt in the soil solution, and produces a suite of effects identical to those of water stress caused by drought. Later, there may be an additional effect on growth; if excessive amounts of salt enter the plant they will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence. This will reduce the amount of assimilate that the plant can produce, and a reduction in the assimilate transported to the growing tissues may further limit growth. This is the second phase of the growth response, and is the phase that clearly separates species and genotypes that differ in the ability to tolerate saline soil.

Thus, growth is limited predominantly by osmotic stress, but in species that have a high rate of salt uptake, or cannot compartmentalize salt effectively in vacuoles, salt-specific effects develop with time, impose an additional stress on the plant through failing capacity to produce photoassimilate, and give rise to the categories of 'salt-sensitive' and 'salt-tolerant'. This implies that any improvement in drought resistance would make a plant more adapted to saline soil. However, the processes that adapt a plant specifically to saline soil involve the regulation of the uptake and compartmentation of salt, to delay as long as possible the time when it accumulates to toxic levels in leaves that are actively photosynthesizing. Breeding or genetic engineering of plants better adapted to saline soil should focus on these processes.

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